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TECHNICAL MANUSCRIPT 163

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TECHNICAL MANUSCRIPT 163

EXPOSURE OF GUINEA PIGS TO X-IRRADIATION AND *P. TULARENSIS* OF REDUCED VIRULENCE

John E. Nutter

Henry T. Eigelsbach

Medical Bacteriology Division
DIRECTOR OF BIOLOGICAL RESEARCH

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ABSTRACT

Studies were conducted to determine and evaluate potential changes in susceptibility, resistance to infection, serology, and subsequent immunity in the guinea pig irradiated before or after respiratory exposure to normally innocuous doses of P. tularensis strain 38A (avirulent) or LVS (live vaccine strain). A 1000-KVP X-ray unit was used for irradiation and it was established that 140 R constituted the maximal sublethal dose for the 325- to 375-g, male, Hartley strain, guinea pig. Although all control animals administered either 140 R of irradiation or P. tularensis via the respiratory route survived, fatalities occurred in animals receiving the combination; greatest mortality was observed in guinea pigs irradiated prior to respiratory exposure. Exposure of irradiated animals to 38A or LVS did not modify the classical leucopenia normally observed following irradiation; nonirradiated animals exposed to LVS or 38A exhibited no marked change in blood picture. Irradiated as well as nonirradiated guinea pigs produced agglutinins after exposure to LVS. In contrast, fewer irradiated animals that received 38A, produced agglutinins than did nonirradiated animals exposed under the same conditions.

EXPOSURE OF GUINEA PIGS TO X-IRRADIATION AND *P. TULARENSIS* OF REDUCED VIRULENCE

Considerable information is available on the effect of large doses of ionizing radiation on immunity as well as on endogenous and exogenous infection. However, relatively little is known concerning the consequences of sublethal irradiation and exposure to exogenous microorganisms usually incapable of producing overt disease. The objective of this study was to determine and evaluate the effect of sublethal X-irradiation on the reaction of the guinea pig to normally innocuous vaccination with attenuated *Pasteurella tularensis*. Studies included investigation of hematological, and serological responses as well as the ultimate fate of the guinea pig to combined radiological and biological exposure; also, the immunity of survivors to subsequent challenge with highly virulent *P. tularensis* strain SCHU S4 was evaluated. In addition, the influence of various relative time intervals between radiological and biological exposure on the response of the host was investigated.

A 1000-KVP X-ray unit was used for irradiation. Whole body exposures were made in a plastic wheel cage at a distance of 100 cm; dosage rates ranged from 56 to 73 röntgens (R) per minute. It was established that 140 R of whole body irradiation constituted the maximum sublethal dose for the 325- to 375-gram male, Hartley strain, guinea pig. This dosage was selected as the standard. Avirulent strain 38A and the live vaccine strain (LVS) were used for respiratory exposure. In a series of two tests, groups of 20 animals, including a control group that was not irradiated, were permitted to inhale approximately 10^6 viable cells of 38A or LVS contained in a small particle aerosol. Another control group consisted of animals that were irradiated but not exposed to 38A or LVS. The relative time intervals for the irradiation were: 12, 6, 3, and 1 day prior to respiratory exposure to the bacteria and 2- to 4-hours, 1 day, and 3 days following.

Results 30 days after respiratory exposure revealed that certain combinations of irradiation and exposure to either 38A or LVS proved fatal to the guinea pig. A summary of the data on animals exposed to LVS is presented in Table I. Animals irradiated three days prior to inhalation of LVS showed the highest mortality response. Deaths occurred in animals irradiated as early as six days prior to exposure or as late as one day after. In this series of tests one of 40 irradiated controls died. All nonirradiated animals survived.

TABLE I. GUINEA PIG MORTALITY AFTER X-IRRADIATION AND RESPIRATORY EXPOSURE TO PASTEURELLA TULARENSIS STRAIN LVS

Time of Irradiation with Respect to Bacterial Exposure, Days	No. Dead / No. Exposed	Per Cent Dead
-12	0/20	0
-6	2/20	10
-3	10/40	25
-1	7/40	17.5
+2 to 4 Hours	0/40	0
+1	2/20	10
+3	0/20	0
Nonirradiated	0/20	0
Irradiated Only	1/40	2.5

Data presented in Table II show that fatalities also occurred in animals exposed to 38A 12 days before to 1 day after irradiation; however, only 7.5 per cent mortality occurred in the group irradiated three days prior to exposure in contrast to 25 per cent mortality when comparable animals were exposed to LVS.

Deaths in irradiated animals occurred three to 17 days after exposure to LVS or 38A; the majority of the animals succumbed during the second week after exposure. P. tularensis was cultured from 13 of 21 irradiated guinea pigs dying after exposure to LVS and from three of ten irradiated animals inhaling 38A.

TABLE II. GUINEA PIG MORTALITY AFTER X-IRRADIATION AND RESPIRATORY EXPOSURE TO PASTEURELLA TULARENSIS STRAIN 38A

Time of Irradiation with Respect to Bacterial Exposure, Days	No. Dead / No. Exposed	Per Cent Dead
-12	1/20	5
-6	0/20	0
-3	3/40	7.5
-1	2/40	5
+2 to 4 Hours	3/40	7.5
+1	1/20	5
+3	0/20	0
Nonirradiated	0/40	0
Irradiated Only[a]	1/40	2.5

a. These data also presented in Table I.

Animals dying within ten days exhibited multiple subcutaneous, intestinal, and peritoneal hemorrhages. In addition, animals exposed to LVS demonstrated petechial hemorrhages in the lung; whereas, animals exposed to 38A did not exhibit this pathology. The subcutaneous intestinal, and peritoneal hemorrhages were more marked in LVS animals than those observed in the animals exposed to 38A. Several petechial hemorrhages were present on the uncut pleural surface of the lungs, and this observation constituted the only major difference in gross pathology observed between animals exposed to 38A or LVS. In contrast, only hemorrhagic lungs were observed in animals dying later than the tenth day following exposure to 38A or LVS. Animals irradiated at the different relative time intervals, with respect to bacterial exposure, and succumbing to the combination could not be differentiated on the basis of gross pathology.

Total leucocyte and differential counts were made from four animals of each irradiation-organism combination twice before irradiation and on alternate days thereafter. The absolute numbers of granulocytes and

lymphocytes-monocytes per cu mm of blood was plotted against time after irradiation for the various irradiation-organism combinations. In general, animals subjected to the combined exposure exhibited the same pattern of leucocyte response as did the irradiation controls (Figure 1). Data plotted were obtained on animals irradiated three days prior to respiratory exposure to LVS. Within two days after irradiation, the number of circulating lymphocytic-monocytic cells of irradiated controls and animals both irradiated and exposed to LVS declined abruptly and remained depressed throughout the observation period of 33 days after irradiation. The number of circulating lymphocytic-monocytic cells were at approximately the point of maximum depression when animals were exposed to LVS.

A more gradual decline in the number of granulocytes in irradiated animals and in animals both irradiated and exposed to LVS was observed. The granulocyte count was not appreciably reduced at the time the test group was exposed to LVS. Maximal depression of the granulocyte count was observed approximately 14 days after irradiation of controls and approximately 10 days after irradiation of animals subsequently exposed to LVS. Gradual return to preirradiation levels was observed within an additional seven days for irradiated-LVS animals and within ten days for irradiated controls. The same general pattern was evident in all irradiated animals regardless of the relative time of radiological and biological exposures or of the strain of P. tularensis. In contrast, nonirradiated animals exposed to 38A or LVS demonstrated a gradual increase in both cell types.

Following the initial 30 day observation period, sera of a representative number of surviving animals were tested serologically for P. tularensis agglutinins. Data on irradiated guinea pigs exposed to LVS are presented in Table III. All animals produced agglutinins regardless of when irradiated. However, when the modal titer values were compared, there was evidence of a trend toward slightly lower titers in all irradiated animals except those irradiated 12 days before exposure to LVS.

Data presented in Table IV show that only 30 per cent of the nonirradiated controls exposed to 38A gave a positive agglutinin titer. The per cent of irradiated animals showing titers ranged from zero in the group irradiated six days before exposure to 40 in the group exposed one day after exposure. The mode of titers in irradiated animals showing positive serology ranged from 1:80 to 1:320; whereas, the mode of the titers observed in nonirradiated animals was 1:160.

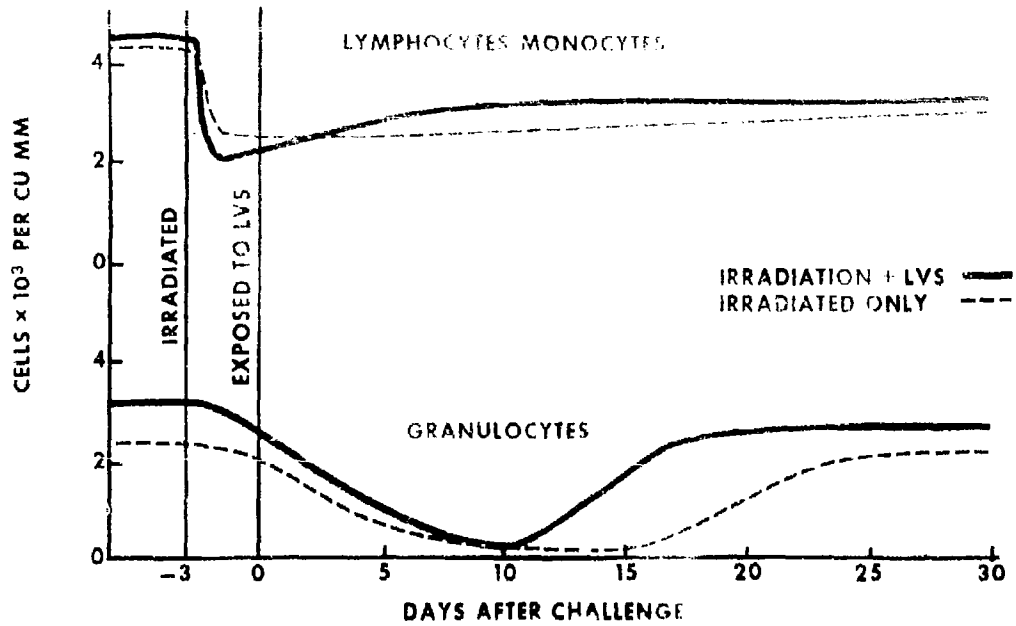

Figure 1. Leucocytic Response of Guinea Pigs to Sublethal X-Irradiation and Pasteurella tularensis Strain LVS.

TABLE III. AGGLUTININ TITERS OF IRRADIATED GUINEA PIGS EXPOSED TO P. TULARENSIS STRAIN LVS

Time of Irradiation with Respect to Bacterial Exposure, Days	Mode
-12	1:320
-6	1:160
-3	1:80
-1	1:160
+2 to 4 Hours	1:160
+1	1:160
+3	1:160
Nonirradiated	1:320

TABLE IV. AGGLUTININ TITERS OF IRRADIATED GUINEA PIGS EXPOSED TO PASTEURELLA TULARENSIS STRAIN 38A

Time of Irradiation with Respect to Bacterial Exposure, Days	Per Cent Showing Agglutinins	Mode of Titer in Animals Showing Positive Serology
-12	10	1:160
-6	0	
-3	17	1:80
-1	11	1:80
+2 to 4 Hours	16	1:160
+1	40	1:320
+3	30	1:80
Nonirradiated	30	1:160

To determine the immunity afforded irradiated animals by respiratory exposure to LVS or strain 38A, survivors were challenged subcutaneously with 10^3 cells of strain SCHU S4 30 days after the aerogenic vaccination with live organisms. Neither nonirradiated controls nor irradiated animals exposed to 38A exhibited resistance to the SCHU S4 challenge. In contrast, as shown in Table V, irradiated animals exposed to LVS and subsequently challenged with SCHU S4 exhibited immunity; moreover, the degree of immunity developed by these animals was only slightly lower than that observed in nonirradiated animals vaccinated in the same manner.

TABLE V. IMMUNOGENICITY OF LIVE TULAREMIA VACCINE FOR THE IRRADIATED GUINEA PIG

Time of Irradiation with Respect to Aerogenic Vaccination with Strain LVS, Days	Per Cent Survival 30 Days after SC Challenge with 10^3 Cells of Strain SCHU S4
-12	60
-6	59
-3	53
-1	59
+2 to 4 Hours	45
Nonirradiated Vaccinated	70
Nonirradiated Nonvaccinated	0

In summary, it was demonstrated that maximal sublethal whole-body X-irradiation of the guinea pigs before or after respiratory exposure to normally innocuous P. tularensis strains LVS or 38A may result in death. Classical leucopenia appeared in irradiated controls and in irradiated animals exposed to LVS or 38A. Irradiation of the guinea pig before or after exposure to LVS or 38A did not affect appreciably the production of P. tularensis agglutinins nor did irradiation prevent the development of resistance to SCHU S4 challenge ordinarily afforded by aerogenic vaccination with LVS.